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(54) Title: DIETARY SUPPLEMENT FOR POST-MENOPAUSAL WOMEN

(57) Abstract

Bone and cardiovascular health can be maintained by the routine administration of the dietary supplements described herein. A dietary supplement of this invention comprises calcium, phytoestrogen and vitamin D present in amounts sufficient to minimize bone loss in a post-menopausal woman; and dietary fiber, vitamin B₁₂, vitamin B₆ and folic acid present in amounts sufficient to reduce total serum cholesterol and low density lipoprotein cholesterol. The dietary supplement and methods are also useful for women lacking their ovaries or having defective ovaries.

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DIETARY SUPPLEMENT FOR POST-MENOPAUSAL WOMEN

RELATED APPLICATIONS

This application claims priority to U.S. Serial No. 09/100,388, filed June 19, 1998, the contents of which are incorporated herein by reference.

5 BACKGROUND OF THE INVENTION

Post-menopausal women are at risk of osteoporosis and coronary heart disease (CHD), among other health conditions. Proper nutritional support for post menopausal women may mitigate or reduce risk factors that result in disease.

Coronary heart disease is a major cause of death in women. It claims the 10 lives of nearly 250,000 women per year, most of whom are post-menopausal. Although generally not manifest until the post-menopausal stage, CHD develops over decades. Well established risk factors for CHD include elevated plasma cholesterol levels and abnormal glucose metabolism. Also implicated in the development of CHD are elevated homocysteine levels and the effects of free 15 radicals. Phytoestrogens, antioxidants, chromium and folic acid have been shown to mitigate these risk factors.

Osteoporosis is associated with the aging process and predominantly affects women. It is characterized by diminished bone density, which results in increased bone fractures and vertebral column collapse. Bone loss begins around about age 20 35. This loss accelerates during the menopause, which generally occurs around age 45 to 55. Osteoporosis develops over decades and is related to peak bone mass, as well as to the degree of bone loss. Adequate calcium intake limits the development of osteoporosis. Moreover, certain vitamins and minerals enhance calcium absorption and utilization.

25 SUMMARY OF THE INVENTION

The invention pertains to a dietary supplement for the purpose of supplementing the micronutrient needs of post-menopausal women for the prevention or reduction of osteoporosis and osteoporetic fractures associated therewith, and coronary heart disease, by retarding bone loss and improving the 30 cardiovascular health of the post-menopausal woman. Bone and cardiovascular

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health can be maintained by the routine administration of the dietary supplements described herein. A dietary supplement of this invention comprises phytoestrogen, calcium and vitamin D present in amounts sufficient to minimize bone loss in a post-menopausal woman; dietary fiber, vitamin B₁₂, vitamin B₆, and folic acid present in amounts sufficient to reduce total serum cholesterol and low density lipoprotein cholesterol; and optionally physiologically acceptable vehicle(s). The dietary fiber can be any soluble fiber (e.g., derived from yeast or plant, such as oat and barley) that ferments in the colon. Alternatively, the dietary fiber can be β -glucans which can be soluble in part and insoluble in part, such as whole yeast glucan particles which are most preferred. Members of the isoflavone class of phytoestrogens (e.g., genistein, daidzein and glycitein) are particularly preferred.

The dietary supplements of this invention may be formulated as a tablet, capsule, powder, suspension, gel or liquid, and are preferably formulated for a single or multiple daily administration. Alternatively, the nutritional supplement can be provided in a variety of forms, such as baked goods, puddings, confections, snack foods, ice cream, frozen confections and novelties, dietary beverages, or non-baked extruded foods such as dietary bars.

The invention also pertains to methods for supplementing the dietary needs and preventing or reducing health risks associated with bone loss and cardiovascular disease in post-menopausal women. The method comprises administering to a post-menopausal woman an effective amount of the dietary supplement of this invention preferably throughout the remainder of the woman's life, to prevent or reduce bone loss and osteoporotic effects associated therewith; to lower serum cholesterol levels; to reduce levels of low density lipoprotein cholesterol and elevate high density lipoprotein cholesterol; or to prevent or reduce the risk of coronary heart disease.

The dietary supplements and the methods of this invention can also be used for women who have defective nonfunctioning ovaries or who lack ovaries since they have similar health risks due to hormonal changes, for instance.

DETAILED DESCRIPTION OF THE INVENTION

The dietary supplement is formulated to supplement the increased nutritional needs of post-menopausal women for the prevention or reduction of the risk of coronary heart disease and osteoporosis throughout this stage of a woman's life. For purposes of this invention, "post-menopause" as the term is used herein is characterized by complete cessation of ovarian function and an increase in the incidence of osteoporotic fracture and coronary heart disease. The dietary supplemental comprises calcium, vitamin D and phytoestrogens present in amounts

sufficient to minimize bone loss in a post-menopausal woman; and vitamin B₁₂, vitamin B₆, folic acid and dietary fiber present in an amount sufficient to reduce total serum cholesterol and low density lipoprotein cholesterol; and optionally in an admixture with physiologically acceptable vehicle(s). In a preferred embodiment, a 5 dietary supplement, formulated in single or multiple units, provides from about 1 mg to about 2000 mg calcium per day; from about 1 µg to about 20 µg vitamin D per day; from about 1 mg to about 100 mg isoflavone per day; from about 1 g to about 50 g β-glucan per day; from about 1 µg to about 2000 µg vitamin B₁₂ per day; from about 1 mg to about 100 mg vitamin B₆ per day; and from about 1 µg to about 1000 10 µg folic acid per day. In another embodiment, a dietary supplement, formulated in single or multiple units, provides from about 500 mg to about 1500 mg calcium per day; from about 5 µg to about 13 µg vitamin D per day; from about 20 mg to about 60 mg isoflavone per day; from about 1.5 µg to about 4.0 µg vitamin B₁₂ per day; from about 1.6 mg to about 4.6 mg vitamin B₆ per day; and from about 180 µg to 15 about 800 µg folic acid per day.

The invention also pertains to a method for supplementing the dietary needs and preventing or reducing life stage associated health risks in post-menopausal women. This method comprises administering (preferably on a daily basis) to a post-menopausal woman an "effective amount" of the dietary supplement of the 20 invention throughout that life stage. The term "effective amount", as used herein, means an amount of the dietary supplement and components contained therein (e.g., either singly or in combination) sufficient to achieve a desired effect, i.e., to prevent or reduce certain health risks in post-menopausal women. The methods of this invention and the term "effective amount" is intended to embrace therapeutic and 25 prophylactic treatment. The dietary supplements of this invention are not meant to replace a well-balanced diet, but are instead intended to supplement a prudent diet. Unlike known broad-based dietary supplements, this invention does not merely provide the prescribed Recommended Daily Allowance (RDA) of micronutrients, but rather is geared to emphasize the disease prevention properties of micronutrient 30 supplementation. Thus, cumulative beneficial and preventive effects should be achieved by dietary supplementation with the appropriate dietary supplement of the invention.

The dietary supplements of this invention contain vitamins, minerals and other compounds that are specifically included in effective amounts to address 35 common health concerns that arise during post-menopause. Moreover, the dietary supplements are formulated to prevent or to lessen the risk of osteoporosis, osteoporotic fractures associated therewith, and coronary heart disease, which are

generally manifest in the post-menopause life stage. The dietary supplement and the methods of this invention can also be used for women who lack ovaries or who have defective, nonfunctioning ovaries (e.g., hormone levels below normal).

Each of the components that are incorporated into the dietary supplement are 5 described in detail below under separate headings. Briefly, calcium, vitamin D and phytoestrogen are incorporated into the dietary supplement to mitigate bone loss in the post-menopausal woman. Dietary fiber, vitamin B₁₂, vitamin B₆ and folate are intended to prophylactically reduce the risks of coronary heart disease, for instance by reducing serum cholesterol and low density lipoprotein cholesterol and raising levels 10 of high density lipoprotein cholesterol.

CALCIUM AND VITAMIN D

The presence of calcium and vitamin D in the dietary supplement of this invention is intended to retard the rate at which bone degrades, thereby minimizing the onset and debilitating effects of osteoporosis. Osteoporosis is the nonvolitional 15 loss of bone that results in an increased susceptibility to fracture, especially of the wrist, lumbar spine and hip region. At menopause, there is an increase in the amount of calcium lost in the urine (Christopher, B.E., "Calcium and Osteoporosis", *Nutrition* 13:664-686 (1997). In addition, the absorption of calcium may be diminished. These two factors coupled together indicate that the intake of calcium 20 should increase with age to at least 1,200 mg per day. Studies using calcium to retard bone loss in aging women suggest that treatment with calcium slowed the rate of bone loss from 1.0% per year (controls without calcium) to 0.014% in the treatment group (not significantly different). Vitamin D deficiency in the elderly also contributes to bone loss. The form of calcium supplementation has also been 25 shown to effect the rate of bone loss. For example, calcium maleate has been shown to work better than calcium carbonate (Dawson-Hughes, *N. Engl. J. Med.*, 323:878-83 (1990)).

The amount of calcium included in the dietary supplements of this invention will depend, in part, on the physical and nutritional condition of the post- 30 menopausal woman, whether the individual is undergoing hormone replacement therapy, as well as the type of calcium used. The amount of calcium will be from about 1 to about 2,000 mg per day; preferably 500 to 1,500 mg per day, with 1,200 mg being optimal as recommended by the Institute of Medicine in the Dietary Reference Intake Guideline of 1997. The calcium may be derived from calcium 35 carbonate, calcium citrate, calcium phosphate, calcium maleate, calcium lactate

maleate, calcium lactate-gluconate, or a combination thereof; with calcium citrate or calcium maleate being most preferred.

Vitamin D (1,25-dihydroxyvitamin D₃) when administered with calcium or calcitonin has been shown to prevent bone loss in women who had a prolonged use 5 of corticosteroids, which had led to bone loss in the lumbar spine (Sambrook et al., *N. Engl. J. Med.*, 328:1747-52 (1993)). Vitamin D is incorporated in the dietary supplement as vitamin D₂ (ergocholecaliferol); vitamin D₃ (cholecalciferol, calcitriol), calcifediol [25(OH)] or combination thereof. The dietary supplement should provide from about 1 to about 20 micrograms per day of vitamin D; 10 preferably from about 5 to about 13 micrograms per day (400 to 800 I.U.). Most preferably, vitamin D will be present in the dietary supplement in the recommended daily allowance (RDA) of about 5 micrograms per day.

PHYTOESTROGENS/ISOFLAVONES

Phytoestrogens and more particularly isoflavones are included in the dietary 15 supplements of this invention. These compounds have been demonstrated by clinical trials to modulate the menstrual cycle, reduce menopausal symptoms, and lower LDL cholesterol (LDL-C) levels. *J. Nutr.*, 1996, 126(1):161-7; *N. Engl. J. Med.*, 1995, 333:276-82. Epidemiologic observations indicate women in countries where diets are high in phytoestrogen (averaging about 40-50 mg/day) have a 20 decreased incidence of breast cancer, menopausal symptoms and osteoporosis. *Nutr. Cancer*, 1994, 21:113-131; *J. Nutr.*, 1995, 125; 757S-770S; *Am. J. Clin. Nutr.* 1995, 62: 645. Animal studies have provided a biological basis for these observations. *J. Ster. Biochem & Mol. Biol.*, 1992, 41 (3-8): 331-7; First International Symposium on the role of Soy and Preventing and Treating Chronic Disease (1994), Speaker. 25 Abstracts. There is increasing evidence that the beneficial effects of soy are attributable to its isoflavone content. Isoflavones or isoflavonoids are a class of phytoestrogens derived from plants, commonly soy beans. The plant source is not critical, however, soybeans are an excellent source of isoflavones and are particularly abundant in genistein. Some phytoestrogens, such as genistin, glycitin 30 and diadzin contain a glycosidic moiety, whereas other phytoestrogens are nonglycosylated, genistein, glycinein and diadzein. Phytoestrogens obtained from a single plant generally are a mixture of both glycosylated and nonglycosylated forms. The dietary supplements of the invention generally contain a mixture of phytoestrogens; however, purified phytoestrogens may be used.

35 Daidzein is a metabolite of ipriflavone which has been shown to increase bone mass. Animal and clinical data suggest that diets containing soy protein or the

isolated isoflavones cause greater bone density, greater bone mechanical strength, and reduced calcium in the urine. It is believed that soy and its isoflavones cause more calcium to be absorbed by the body and bone calcium absorption to be enhanced. Soy isoflavones have estrogenic effects, which are mediated through

5 estrogen receptors. In general, the soy isoflavones are weaker than estradiol and diethylstilbestrol. Most notably, genistein is known to be an estrogen analog and will bind to estrogen receptors with the affinity of 0.2% of estradiol, the major circulating estrogen (Erdman JW, Potter SM. Soy and bone health. The Soy Connection volume 5, 1997). In some instances, the isoflavones have antiestrogenic

10 effects rather than estrogenic effects. It usually depends upon the particular tissue how the isoflavones behaves. In the case of bone, isoflavones have estrogenic action or are estrogen agonists and should improve bone health.

Sixty-six postmenopausal, hypercholesterolemic women were randomized to the NCEP Step 1 diet (National Cholesterol Education Program) and isolated soy

15 protein with moderate isoflavones, soy protein with high amounts of isoflavones, or casein (control) (Erdman JW, Potter SM. Soy and bone health. The Soy Connection. 1997; volume 5, page 1). The test period lasted 24 weeks. Bone mineral measurements of the lumbar spine, the proximal femur, and the total body were measured by DEXA (dual-energy X-ray absorptionmetry) at the beginning of the

20 study and at the end (24 weeks). Only the lumbar spine bone mineral content and density increased (2%) in the high isoflavones group, and there were no changes in the other groups. The spine is more sensitive to estrogen due to its higher content of trabecular bone. This type of bone undergoes remodeling at a much more rapid rate than the hip, which contains a higher proportion of cortical bone. The 2% increase

25 in a short period was impressive.

Later, John Crouse, III, M.D., professor of internal medicine at Wake Forest University evaluated the effect of soy protein containing different amounts of isoflavones on serum cholesterol and LDL-C levels in mildly hypercholesterolemic patients (American Heart Association, 38th Annual Meeting of Cardiovascular

30 Disease, Epidemiology, and Prevention, March 20, 1998). Subjects had mean cholesterol levels of 241 mg/dL and LDL-C of 164 mg/dL and included men (94), pre-menopausal women (24), and post-menopausal women (38). Subjects received 25 g of soy protein with 4, 25, 42, or 58 mg of the isoflavones. The higher the concentration of the isoflavones administered, the greater the reduction in both total

35 cholesterol and LDL-C. These patients had a 10% reduction in both values after 9 weeks. This is the first report demonstrating that the isoflavones and not the soy protein have the lipid-lowering effect.

Soy protein is proposed to lower serum cholesterol by lowering LDL-C through enhanced degradation by small peptides formed during digestion of soy protein. In addition, the soy isoflavones, genistein and daidzein are antioxidants and can cause a significant inhibition of oxidation of LDL. These isoflavones appear to

5 exert this effect on the LDL-C in an aqueous medium inside the arterial wall rather than on LDL-C in the plasma. The isoflavone, genistein, is a specific inhibitor of tyrosine kinase, an enzyme important in the mechanism leading to platelet aggregation and blood clotting. Ingestion of this isoflavone or soy protein may help to decrease thrombosis and prevent mortality from cardiovascular disease

10 (Kurowska EM. Soy and reduced risk of cardiovascular disease. The Soy Connection, vol. 5, page 1, 1997).

Soy may also prevent breast cancer and hot flashes that occur during menopause. Genistein and daidzein have been shown to inhibit human breast cancer and prostate cancer cell lines in cultures by mechanisms independent of steroid

15 binding (ADM, Technical Report, 1998). Isoflavones may exert their effect by inhibiting tumor growth and inducing cell maturation. Chinese women who are pre-menopausal have a reduced incidence of breast cancer compared to women in other cultures where soy is not consumed regularly. This phenomenon has been shown to be true for women in Australia who eat a high soy diet and women in other parts of

20 Southeast Asia.

Women undergoing menopause may have fewer and less severe hot flashes (Barnes S., Kim H., Soy isoflavone, estrogens, and growth factor signaling. The Soy Connection volume 6, 1998). The weak estrogenic effects of isoflavones from soy may help relieve symptoms. Hot flashes decreased by 45% after 12 weeks of 60

25 g of soy powder (40 g protein) in Italian women.

Based upon the foregoing, phytoestrogen levels of from about 1 to about 100 mg per day, preferably from about 20 to about 60 mg per day, and most preferably about 50 mg per day, provide women with phytoestrogen levels that are physiologically effective and may be used to supplement women's micronutrient

30 needs during post-menopause. The phytoestrogen can be selected from the group consisting of genistin, glycitin, diadzin, genistein, glycinein, diadzein and combinations thereof. A preferred mixture of phytoestrogen may be obtained commercially such as Novasoy (genistein: daidzein: glycinein in a ratio of 1.1:1:0.2) from Archer Daniels Midland (ADM).

DIETARY FIBER

Dietary fiber suitable for reducing total serum cholesterol and low density lipoprotein cholesterol is also incorporated in the dietary supplement of the present invention. The dietary fiber can be any soluble fiber (e.g., derived from yeast or plant, such as oat and barley) that ferments in the colon. Examples of suitable dietary fiber, include but are not limited to, nondigestible polysaccharides, plant cell walls, carageenans, mucilages, glucans (e.g., β -glucans), gums, agar, some hemicelluloses, psyllium and pectin. Alternatively, the dietary fiber can be a β -glucan which is soluble in part and insoluble in part, such as whole yeast glucan particles which are most preferred. Yeast β -glucan has been shown to reduce serum cholesterol and LDL-C concentrations and raise high density lipoprotein cholesterol levels. See U.S. Patent No. 4,962,094; the entire teachings of which are incorporated herein by reference. Hypercholesterolemic subjects (> 240 mg/dL) were enrolled in a twelve week study after a three week wash-in period. Subjects received 15 grams of yeast β -glucan (FibercelTM, Alpha-Beta Technology, Inc.; whole yeast glucan particles having an *in vivo* glucan morphology) in orange juice for eight weeks. This was followed by a four week period without fiber supplementation. The total cholesterol levels were significantly lower at week seven (approximately 9%, $p = 0.05$) and at week eight (approximately 7%, $p = 0.05$). These levels returned to normal after four weeks without the fiber. The LDL-C was reduced at week six (approximately 7%, $p < 0.05$) and remained significantly lower than baseline throughout the fiber supplementation. It normalized after four weeks without the fiber. The HDL-C rose significantly after eight weeks of supplementation (15%, $p < 0.05$) and remained significantly higher even after four weeks without fiber supplementation ($p = 0.02$). The fiber was well tolerated and patients remained weight stable, indicating that the changes in serum lipids were not attributable to weight loss.

The amount of dietary fiber included in the dietary supplement of this invention will vary depending upon the source and type of fiber incorporated therein, but in any event, should be an amount sufficient to reduce total serum cholesterol and low density lipoprotein cholesterol. Dietary fiber can be incorporated into the dietary supplement in amounts of from about 1 g to about 50 gm/day; with preferably from about 5 g to about 20 gm/day; most preferably 15 gm/day. A particularly preferred dietary is yeast β -glucan, such as that described in U.S. Patent No. 4,962,094 (FibercelTM).

B VITAMINS AND FOLATE

Vitamin B₁₂ (cyanocobalamin), vitamin B₆ (pyridoxine) and folic acid are included in the dietary supplement of this invention. These elements act synergistically to reduce serum homocysteine; high levels of which are associated with coronary heart disease. *Am. J. Clin. Nutr.*, 1992, 55:131-138; *New Eng. J. Med.*, 1992, 32:1832-1835; *Am J. Clin. Nutr.*, 1989, 50:353-358. The amount of vitamin B₁₂ is from about 1 to about 2,000 mg/day, preferably from about 1.5 to about 4.0 µg/day, with 2.0 µg/day being optimal. The amount of vitamin B₆ present in the dietary supplement is from about 1 to about 100 mg/day, preferably from about 1.6 to about 4.6 mg/day, with 4.6 mg/day being most preferred (RDA = 1.6 mg). Folic acid is provided in the dietary supplement as it has been shown to have beneficial cardiac effects and to decrease the risk of cervical dysplasia. *Scand. J. Clin. Lab Invest.*, 1988, 48:215-221. The amount of folate present in the dietary supplement is from about 1 to about 1,000 µg/day, preferably from about 80 to about 800 µg/day, and most preferably about 696 µg/day (RDA = 180 µg). It is desirable to incorporate at least two times RDA amounts of B₆ and folate into the dietary supplement of this invention.

Recently, Rimm *et al.* reported that folate and B₆ should be ingested in excess of the Recommended Dietary Allowance to prevent coronary heart disease (CHD) in women (*JAMA*, 279:359-364, 1998). The study included 80,082 women followed over a 14-year period during which time 281 cases of fatal heart attacks occurred. The risk of CHD was reduced among women who regularly used multiple vitamins, the major source of folate and B₆ (RR=0.76; 95% CI, 0.65-0.90). The relative risk of developing cardiovascular disease between the highest and lowest quintile for folate and vitamin B₆ was 0.55. The best results were seen in women who took 696 µg per day of folate and 131 mg per day of B₆.

FORMULATION AND UTILITY OF DIETARY SUPPLEMENTS

The dietary supplements of the invention are intended to be orally administered daily. Preferably, they are formulated for single daily administration, but may be formulated in multiple portions or as time release compositions for more or less frequent administration; for example, the dietary supplement may be formulated as two tablets for twice daily administration. For reasons of size (ease of swallowing) or improved bioabsorption or utilization (e.g., before or after a meal or before sleep), a given dosage may be divided into two, three, or more tablets (or capsules, etc.). A daily dosage may be administered as one tablet, as two tablets taken together, or as two tablets taken separately (e.g., one in the morning and one in the evening). The recommended daily amounts of each ingredient, as described

above, serve as a guideline for formulating the dietary supplements of this invention. The actual amount of each ingredient per unit dosage will depend upon the number of units daily administered to the post-menopausal woman. This is a matter of product design and is well within the skill of the dietary supplement formulator.

5 The dietary supplements of the present invention may be formulated using any pharmaceutically acceptable forms of the vitamins, minerals and other nutrients discussed above, including their salts. They may be formulated into capsules, tablets, powders, suspensions, gels or liquids optionally comprising a physiologically acceptable carrier, such as but not limited to water, milk, juice,

10 starch, vegetable oils, salt solutions, hydroxymethyl cellulose, carbohydrate. The dietary supplements may be formulated as powders, for example, for mixing with consumable liquids, such as milk, juice, water or consumable gels or syrups for mixing into other dietary liquids or foods. The dietary supplements of this invention may be formulated with other foods or liquids to provide premeasured supplemental

15 foods, such as single servings bars, for example.

The dietary supplement can be made in a variety of forms, such as baked goods, (e.g., cookies, brownies, fudge, cake, breads, biscuits, crackers,), puddings, confections, (i.e., candy), snack foods (e.g., pretzels, chips), dietary beverages, ice cream, frozen confections and novelties, or non-baked, extruded food products such

20 as bars. The preferred form is a non-baked extruded nutritional bar.

The dietary supplement can also contain other ingredients such as one or a combination of other vitamins, minerals, antioxidants, fiber and other dietary supplements. Selection of one or several of these ingredients is a matter of formulation design, consumer and end-user preference . The amount of these

25 ingredients added to the dietary supplements of this invention are readily known to the skilled artisan and guidance to such amounts can be provided by the U.S. RDA doses for children and adults. Vitamins and minerals that can be added include, but are not limited to, calcium phosphate or acetate, tribasic; potassium phosphate, dibasic; magnesium sulfate or oxide; salt (sodium chloride); potassium chloride or acetate; ascorbic acid; ferric orthophosphate; niacinamide; zinc sulfate or oxide; calcium pantothenate; copper gluconate; riboflavin; beta-carotene; pyridoxine hydrochloride; thiamin mononitrate; folic acid; biotin; chromium chloride or picolinate; potassium iodide; sodium selenate; sodium molybdate; phylloquinone; Vitamin D₃; cyanocobalamin; sodium selenite; copper sulfate; Vitamin A; Vitamin

30 E; vitamin B₆ and hydrochloride thereof; Vitamin C; inositol; Vitamin B₁₂; potassium iodide.

The dietary supplement can comprise one or a combination of antioxidants in therapeutic amounts. Antioxidants suitable for use in this invention include but are

not limited to, vitamin A, vitamin C, vitamin E, β -carotene, zinc, chromium, selenium and herbs, such as ginkgo biloba, ginseng. The amount of antioxidant(s) per unit serving are a matter of design and will depend upon the total number of unit servings of the dietary supplement daily administered to the patient. The total 5 amount of antioxidant(s) will also depend, in part, upon the condition of the patient. Preferably the amount of antioxidant(s) will be a fraction or multiplier of the RDA amounts. For example, the dietary supplement will comprise 50% RDA antioxidants per unit dosage and the patient will consume two units per day.

Flavors, coloring agents, spices, nuts and the like can be incorporated into 10 the product. Flavorings can be in the form of flavored extracts, volatile oils, chocolate flavorings, peanut butter flavoring, cookie crumbs, crisp rice, vanilla or any commercially available flavoring. Examples of useful flavorings include but are not limited to pure anise extract, imitation banana extract, imitation cherry extract, chocolate extract, pure lemon extract, pure orange extract, pure peppermint extract, 15 imitation pineapple extract, imitation rum extract, imitation strawberry extract, or pure vanilla extract; or volatile oils, such as balm oil, bay oil, bergamot oil, cedarwood oil, cherry oil, walnut oil, cinnamon oil, clove oil, or peppermint oil; peanut butter, chocolate flavoring, vanilla cookie crumb, butterscotch or toffee. In a preferred embodiment, the dietary supplement contains cocoa or chocolate.

20 Emulsifiers may be added for stability of the final product. Examples of suitable emulsifiers include, but are not limited to, lecithin (e.g., from egg or soy), and/or mono- and di-glycerides. Other emulsifiers are readily apparent to the skilled artisan and selection of suitable emulsifier(s) will depend, in part, upon the formulation and final product.

25 Preservatives may also be added to the dietary supplement to extend product shelf life. Preferably, preservatives such as potassium sorbate, sodium sorbate, potassium benzoate, sodium benzoate or calcium disodium EDTA are used.

In addition to the carbohydrates described above, the dietary supplement can 30 contain artificial sweeteners, e.g., saccharides, cyclamates, aspartamine, aspartame, acesulfame K, and/or sorbitol. Such artificial sweeteners can be desirable if the dietary supplement is intended for an overweight or obese individual, or an individual with type II diabetes who is prone to hyperglycemia.

To manufacture such a food bar, the liquid ingredients are cooked; the dry 35 ingredients are added with the liquid ingredients in a mixer and mixed until the dough phase is reached; the dough is put into an extruded, and extruded; the extruded dough is cut into appropriate lengths; and the product is cooled. For manufacture of other foods or beverages, the ingredients comprising the dietary supplement of this invention can be added to traditional formulations or they can be

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used to replace traditional ingredients. Those skilled in food formulating will be able to design appropriate foods/beverages with the objective of this invention in mind.

While this invention has been particularly shown and described with
5 references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. A dietary supplement for post-menopausal women or women lacking their ovaries, comprising:
 - 5 calcium, phytoestrogen and vitamin D, present in amounts sufficient to minimize bone loss;
 - 10 dietary fiber, vitamin B₆, vitamin B₁₂ and folic acid, present in amounts sufficient to reduce total serum cholesterol and low density lipoprotein cholesterol; and
 - 15 an optional physiologically acceptable vehicle.
 2. The dietary supplement of Claim 1 wherein the dietary fiber is β-glucan is from barley, oat or yeast.
 3. The dietary supplement of Claim 1 wherein the phytoestrogen is a soy-derived phytoestrogen.
 - 15 4. The dietary supplement of Claim 1 wherein the phytoestrogen is an isoflavone.
 5. The dietary supplement of Claim 1 wherein the isoflavone is selected from the group consisting of genistein, daidzein, glycinein, genistin, glycinin, diadzin and combinations thereof.
 - 20 6. The dietary supplement of Claim 1 wherein the isoflavone is a combination of genistein, daidzein and glycinein.
 7. The dietary supplement of Claim 1 wherein the calcium is derived from calcium carbonate, calcium citrate, calcium phosphate, calcium maleate, calcium lactate maleate, calcium lactate gluconate, or a combination thereof.
 - 25 8. The dietary supplement of Claim 1 wherein said supplement is formulated in tablet, powder, suspension, liquid, capsule or gel form, or dietary bar.
 9. The dietary supplement, wherein said supplement is formulated into a plurality of oral dosage forms for ingestion on a daily basis.

10. A dietary supplement for post-menopausal women or women lacking their ovaries, the dietary supplement formulated in single or multiple units sufficient to provide a daily dosage of from about 1 mg to about 2000 mg calcium per day; from about 1 μ g to about 20 μ g vitamin D per day; from 5 about 1 mg to about 100 mg isoflavone per day; from about 1 g to about 50 g β -glucan per day; from about 1 μ g to about 2000 μ g vitamin B₁₂ per day; from about 1 mg to about 100 mg vitamin B₆ per day; and from about 1 μ g to about 1000 μ g folic acid per day.
11. A dietary supplement for post-menopausal women or women lacking their ovaries, the dietary supplement formulated in single or multiple units sufficient to provide a daily dosage of from about 500 mg to about 1500 mg calcium per day; from about 5 μ g to about 13 μ g vitamin D per day; from 10 about 20 mg to about 60 mg isoflavone per day; from about 1.5 μ g to about 4.0 μ g vitamin B₁₂ per day; from about 1.6 mg to about 4.6 mg vitamin B₆ per day; and from about 180 μ g to about 800 μ g folic acid per day. 15
12. A dietary food bar for post-menopausal women or women lacking their ovaries, comprising:
 - calcium, phytoestrogen and vitamin D, present in amounts sufficient to minimize bone loss;
 - 20 dietary fiber, vitamin B₆, vitamin B₁₂ and folic acid, present in amounts sufficient to reduce total serum cholesterol and low density lipoprotein cholesterol; and
 - an optional physiologically acceptable vehicle.
13. The dietary food bar of Claim 12 wherein the food bar is formulated into a 25 single or multiple units to provide a daily dosage of from about 1 mg to about 2000 mg calcium per day; from about 1 μ g to about 20 μ g vitamin D per day; from about 1 mg to about 100 mg isoflavone per day; from about 1 g to about 50 g β -glucan per day; from about 1 μ g to about 2000 μ g vitamin B₁₂ per day; from about 1 mg to about 100 mg vitamin B₆ per day; and from 30 about 1 μ g to about 1000 μ g folic acid per day.
14. The dietary food bar of Claim 13 wherein the food bar is formulated into a single or multiple units to provide a daily dosage of from about 500 mg to about 1500 mg calcium per day; from about 5 μ g to about 13 μ g vitamin D

per day; from about 20 mg to about 60 mg isoflavone per day; from about 1.5 μ g to about 4.0 μ g vitamin B₁₂ per day; from about 1.6 mg to about 4.6 mg vitamin B₆ per day; and from about 180 μ g to about 800 μ g folic acid per day.

- 5 15. The dietary food bar of Claim 12 wherein the phytoestrogen is a soy-derived phytoestrogen.
16. The dietary food bar of Claim 12 wherein the phytoestrogen is an isoflavone.
17. The dietary food bar of Claim 12 wherein the isoflavone is selected from the group consisting of genistein, daidzein, glycitein, genistin, glycitin, diadzin and combinations thereof.
- 10 18. The dietary food bar of Claim 12 wherein the isoflavone is a combination of genistein, daidzein and glycitein.
19. The dietary food bar of Claim 12 wherein the dietary supplement is formulated into a plurality of oral dosage forms for ingestion on a daily basis.
- 15 20. A method for daily supplementing micronutrients needs of a post-menopausal woman or a woman lacking ovaries, comprising daily administering to the woman a dietary supplement comprising:
 - 20 calcium, phytoestrogen and vitamin D, present in amounts sufficient to minimize bone loss;
 - dietary fiber, vitamin B₆, vitamin B₁₂ and folic acid, present in amounts sufficient to reduce total serum cholesterol and low density lipoprotein cholesterol; and
 - an optional physiologically acceptable vehicle.
- 25 21. A method for reducing the risk of osteoporosis in a post-menopausal woman or a woman lacking ovaries, comprising daily administering to the woman the dietary supplement of Claim 1.
22. A method for preventing or reducing the risk of coronary heart disease in a post-menopausal woman or a woman lacking ovaries, comprising daily administering to the woman the dietary supplement of Claim 1.

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23. A method for reducing serum cholesterol and low density lipoprotein cholesterol concentration in a post-menopausal woman or a woman lacking ovaries, comprising daily administering to the woman the dietary supplement of Claim 1.
- 5 24. A method for raising high density lipoprotein cholesterol concentrations in a post-menopausal woman or a woman lacking ovaries, comprising daily administering to the woman the dietary supplement of Claim 1.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/13676

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 A23L1/30 A23L1/302 A23L1/304 A61K33/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 962 094 A (BISTRAN ET AL.) 9 October 1990 (1990-10-09) cited in the application claim 1; example 5 ---	1-6,8,9, 11,20-24
Y	WO 98 04248 A (ENERGETICS INC.) 5 February 1998 (1998-02-05) page 17, line 13 - line 31; claims page 26, line 7 -page 27, line 12 ---	10,13,14
X	WO 98 03084 A (NUTRICOR INC.) 29 January 1998 (1998-01-29) page 18, line 1 - line 15; table 10 ---	1,3-9, 11,12, 15-24
Y	US 5 603 936 A (MONTE) 18 February 1997 (1997-02-18) examples 14,33 ---	10,13,14
X	US 5 603 936 A (MONTE) 18 February 1997 (1997-02-18) examples 14,33 ---	1-24
		1,4,7,8
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/13676

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CURRENT PROBLEMS IN OBSTETRICS, GYNECOLOGY AND FERTILITY, vol. 19, no. 4, 1996, pages 112-166, XP002119995</p> <p>YEAR BOOK MEDICAL PUBLISHERS, CHICAGO, IL., US</p> <p>ISSN: 8756-0410</p> <p>page 132, right-hand column -page 134, left-hand column</p> <p>page 136, paragraph CALCIUM</p> <p>page 146 -page 147</p> <p>page 149 -page 154</p> <p>page 157, paragraph CONCLUSION</p> <p>-----</p>	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/13676

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 4962094	A 09-10-1990	AT	106193 T	15-06-1994
		AU	643670 B	25-11-1993
		AU	4492489 A	14-05-1990
		CA	2001754 A	28-04-1990
		DE	68915773 D	07-07-1994
		DE	68915773 T	03-11-1994
		EP	0440725 A	14-08-1991
		IE	64707 B	23-08-1995
		IL	92098 A	12-04-1994
		JP	4505997 T	22-10-1992
		MX	168000 B	27-04-1993
		NZ	231176 A	26-05-1997
		WO	9004334 A	03-05-1990
-----	-----	-----	-----	-----
WO 9804248	A 05-02-1998	US	5654011 A	05-08-1997
		US	5807586 A	15-09-1998
		AU	3958797 A	20-02-1998
		CA	2261764 A	05-02-1998
		EP	0934060 A	11-08-1999
-----	-----	-----	-----	-----
WO 9803084	A 29-01-1998	AU	3887597 A	10-02-1998
-----	-----	-----	-----	-----
US 5603936	A 18-02-1997	NONE	-----	-----
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